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Electrophysiology

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Updated date: Jul 13, 2020

An abbreviated version of this protocol was published in eLIFE in May 2016

Gap junction networks in mushroom bodies participate in visual learning and memory in *Drosophila*

DOI: 10.7554/eLife.13238

Detailed protocol

This protocol is based on the whole cell patch method in Wilson, R.I., and Laurent, G. Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the Drosophila antennal lobe. J. Neurosci, 25(40):9069-79, 2005.

Materials and Reagents:

1. Micropipette
2. Dish
3. Sterile Disposable Filter Units
4. NaCl
5. KCl
6. N-Tris (hydroxymethyl) methyl-2- aminoethane-sulfonic acid
7. Trehalose
8. Glucose
9. NaHCO3, 1 mM
10. NaH2PO4
11. CaCl2, and 4 mM
12. MgCl2
13. potassium aspartate
14. HEPES
15. MgATP
16. Na3GTP
17. EGTA
18. Alexa Fluor 568
19. biocytin hydrazide
20. TTX
21. 2-OCT

Equipments

1. Forceps
2. DC power supply
3. Vacuum pump
4. Vapor pressure osmometer (Wesco, 5520)
5. Benchtop pH meter (Orion Aplus,)
6. Flaming/Brown Micropipette Puller (Sutter,P-97)
7. Fluorescence microscope (Zeiss Axioskop2 or Olympus BX61WI microscope)
8. Motorized Micromanipulator System (Sutter, MP-225)
9. Amplifier (Molecular Device, MultiClamp 700b)
10. Digitizer (Molecular Device, Axon Digidata 1440A)

Procedure

1. Prepare the extracellular saline solution (ECS) of Drosophila. Filter the saline by Sterile disposable filter Unit, then keep the saline under 4 ℃.
2. Prepare the innercellular saline solution (ICS) of Drosophila. Keep the saline under -20 ℃ after sub-packing.
3. Chloride the Ag wire by connecting it to the positive pole of the DC power supply and immersing it into NaCl solution (0.9%) and passing a current at a rate of 1 mA/cm2 of surface area until adequately plated. The color of a well plated wire should be light gray. Occasionally reverse the polarity for several seconds while plating. Put the Ag/AgCl wire back to the electrode holder.
4. Prepare the glass micropipette by a Micropipette Puller. Adjust the protocol by following PIPETTE COOKBOOK to get appropriate glass electrodes with resistance of 20–30 MΩ.
5. Dissect the fly brains out and immerse it in extracellular saline solution in a small dish. Remove the sheath covering the target neuron carefully with fine forceps while leave the sheath on the opposite side of the brain. Keep the target neurons on the upward side by attaching the other side to the bottom of the dish.
6. Put the dish with the brain on the upright fluorescence microscope. Locate the target neuron by the fluorescence.
7. Fill the glass electrode with ICS, and mount it on the holder. Open the amplifier and the digitizer. Use pClamp to monitor the resistance of electrode. Add positive pressure to the electrode, then immerse the electrode to the ECS in dish by micromanipulator, and now the resistance of electrode is 20-30 MΩ.
8. Move the electrode to approach target neuron with visual guidance under the bright field microscopy. Poke the target neuron by the electrode to get an appropriate pit. Release the positive pressure and add negative pressure to the electrode promptly, then get giga seal. If failure, repeat step 7-8.
9. Record the membrane potential of the target neuron. Filter the data with a 10 kHz low-pass filter and acquire the data at 20 kHz.

Recipes

ECS: 103 mM NaCl, 3 mM KCl, 5 mM N-Tris (hydroxymethyl) methyl-2- aminoethane-sulfonic acid, 10 mM trehalose, 8 mM glucose, 26 mM NaHCO3, 1 mM NaH2PO4, 1.5 mM CaCl2, and 4 mM MgCl2, adjusted to 275 mOsm. The saline was bubbled with 95% O2/5% CO2 gas for a final pH of 7.3.
ICS: 140 mM potassium aspartate, 10 mM HEPES, 1 mM KCl, 4 mM MgATP, 0.5 mM Na3GTP, 1 mM EGTA, and 100 mM Alexa Fluor 568 or 1% biocytin hydrazide, pH 7.3, adjusted to 265 mOsm.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Liu, Q. , Guo, A. and Yang, X. (2020). Electrophysiology. Bio-protocol Preprint. bio-protocol.org/prep386.
2. Liu, Q., Yang, X., Tian, J., Gao, Z., Wang, M., Li, Y. and Guo, A.(2016). Gap junction networks in mushroom bodies participate in visual learning and memory in *Drosophila*. eLIFE. DOI: 10.7554/eLife.13238